LncRNA FOXD3-AS1 enhances the migration and invasion ability of breast cancer cells through the Wnt/β-Catenin signaling pathway

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Research Article

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Abstract

Objective

To explore the expression, biological function, and mechanism of action of the LncRNA FOXD3-AS1 in breast cancer.

Methods

The expression of LncRNA FOXD3-AS1 was analyzed in the TCGA database using bioinformatics analysis. Moreover, the relative expression of LncRNA FOXD3-AS1 was measured in the human normal mammary epithelial cell line (MCF10A) and three breast cancer cell lines (BT-549, MDA-MB-231, and MCF-7). After siRNA knockdown of FOXD3-AS1 expression in BT-549, MDA-MB-231 cells, alterations in cell proliferation, migration, invasion, and apoptotic capacity were determined by CCK-8, scratch assay, transwell assay, and Annexin V-FITC/PI staining, respectively. Furthermore, changes in the level of the β-Catenin protein were detected using western blot.

Results

TCGA database analysis results relative to adjacent tissues revealed that the expression of LncRNA FOXD3-AS1 was markedly elevated in breast cancer tissues. Besides, results of real-time PCR established that the relative expression of FOXD3-AS1 was significantly higher in the breast cancer cell lines (BT-549, MDA-MB-231, and MCF-7) than in the normal human mammary epithelial cell line (MCF10A) ($P < 0.0001$). Inhibition of FOXD3-AS1 expression by siRNA significantly inhibited the wound healing ability, migration and invasion ability of BT-549 and MDA-MB-231 cells. However, there was no difference in proliferation and apoptosis. The western blot results showed that inhibition of FOXD3-AS1 expression by siRNA significantly inhibited the level of the β-Catenin protein in BT-549 and MDA-MB-231 cells.

Conclusion

The abnormally high expression of LncRNA FOXD3-AS1 in breast cancer may be related to disease progression. LncRNA FOXD3-AS1 can significantly enhance the migration and invasion ability of breast cancer cells, and its mechanism of action may be related to the Wnt/ β-Catenin signaling pathway.

Introduction

Breast cancer is one of the most common malignant tumors in women. In 2020, the incidence rate of breast cancer[1]in women exceeded that of lung cancer, and became the highest cancer incidence rate in the world. In 2020, the number of new cases of breast cancer in the world was 2261419, and the number of deaths was 684996 cases, accounting for 24.5% and 15.5% of the new cancers and deaths of women

respectively. Indeed, breast cancer has become a great threat to women's health. From the perspective of women [2-4] , breast cancer ranks first among disease spectrum and causes of death in most countries in the world. Therefore, seeking effective biomarkers is critical for the prevention and control of breast cancer. Long-chain non-coding RNAs[5](LncRNAs) refer to non-coding RNAs (ncRNAs) with lengths of 200bp and higher. So far, they have proved to regulate various levels of gene expression programs[6-9]: RNA maturation and transport, regulation of chromatin structure, participation in post-transcriptional gene regulation, control of protein synthesis, etc. LncRNAs can exert their biological functions by enabling transcriptional interference to induce chromatin remodeling and histone modifications, regulate alternative splicing patterns, synthesize endogenous siRNA, regulate protein activity, play structural or organizational roles, and alter protein localization. Recently, studies on LncRNA FOXD3-AS1 have reported the aberrant expression of LncRNA FOXD3-AS1 in multiple diseases[10-13].Notably, this LncRNA either promoted or inhibited disease progression through different mechanisms. Thus, LncRNA FOXD3-AS1 could provide novel targets for diagnosing diseases while serving as a biomarker to predict disease prognosis. This study aimed to clarify the role of LncRNA FOXD3-AS1 on the proliferation, migration, invasion, and apoptotic processes in breast cancer cells and to explore the relevant mechanisms of action.

1. Materials And Methods

1.1 Cells and reagents

Human breast cancer cell lines BT-549, MDA-MB-231, MCF-7, and the normal human breast epithelial cell line (MCF10A) were purchased from the Kunming Cell Bank of Type Culture Conservation Committee of the Chinese Academy of Sciences.RPMI 1640 Medium, DMEM Medium, MEM Medium, MEpiCM Medium, and fetal calf serum were purchased from Gibco, USA. The siRNA was purchased from Biotechnology (Shanghai) Co., Ltd. The Lipofectamine RNAiMAX transfection and TRIzol reagents were procured from Invitrogen, USA. The real-time quantitative polymerase chain reaction (real-time fluorescence quantitative polymerase chain reaction, RTFQ-PCR) kit was purchased from Takara, Japan. The Cell Counting Kit-8 (cell counting kit-8, CCK-8) was purchased from Dojindo Corporation, Japan. Annexin V-fluorescein isothiocyanate (fluorescein isothiocyanate, FITC) / propidium iodide (propidium iodide, PI) Apoptosis detection kit was purchased from Jiangsu Kaiki Biotechnology Co., Ltd.

1.2 Cell culture and passage

Human normal breast epithelial cell line MCF10A and human breast cancer cell lines BT-549, MDA-MB-231, and MCF-7 were added to MEpiCM Medium,RPMI 1640 Medium, DMEM Medium, and MEM Medium containing 10% fetal bovine serum, respectively, and cultured in an incubator with 5% CO2 at 37 ℃. After the cells reached 80-90% confluency, cells in good growth condition were subcultured .

1.3 Detection of LncRNA FOXD3-AS1 mRNA expression level

The total RNA of cells and tissues was extracted by the TRIzol reagent and reverse transcribed into cDNA using an RT-PCR kit as per the manufacturer's instructions. With GAPDH as the internal control, the relative expression of LncRNA FOXD3-AS1 was determined and analyzed by the 2-ΔΔCT method. The primer sequence is as follows (Table 1):

Table 1 Sequences of primers used for RT-PCR

Primer name	Sequences (5'-3')
FOXD3-AS1	F: GGT GGA GGA GGC GAG GAT G
	R: AGC GGA CAG ACA GGG ATT GG
GAPDH	F: UGA CCU CAA CUA CAU GGU UTT
	R: AAC CAU GUA GUU GAG GUC ATT

F: forward; R: reverse.

1.4 SiRNA down-regulated the expression of LncRNA FOXD3-AS1 in breast cancer cells 1.4.1 Small interference RNA transfection: According to the full-length LncRNA FOXD3-AS1 sequence, three small interference RNAs (FOXD3-AS1-Homo-135 (si1),FOXD3-AS1-Homo-560 (si2), and FOXD3-AS1-Homo-382 (si3)) were designed and synthesized by the Shanghai Jima Company, and three specific small interference RNAs (si1,si2 and si3) and small interference RNAs against GAPDH (siNC) were transfected using the Lipofectamine RNAiMAX transfection reagent. The sequence is as follows (Table 2):

Table 2 Sequences of siRNA

siRNA name	Sequences (5'-3')
FOXD3-AS1-Homo-135 (si1)	S: GGA GCG GCU UUA AAG AGU ATT
	A: UAC UCU UUA AAG CCG CUC CTT
FOXD3-AS1-Homo-560 (si2)	S: GUG GAC AAA UCC UCC AAG ATT
	A: UCU UGG AGG AUU UGU CCA CTT
FOXD3-AS1-Homo-382 (si3)	S: GGC UAU UGA UUA AUC UAC UTT
	A: AGU AGA UUA AUC AAU AGC CTT
small interference RNAs against GAPDH (siNC)	S: UUC UCC GAA CGU GUC ACG UTT
	A: ACG UGA CAC GUU CGG AGA ATT

S: sense; A: antisence.

1.4.2 Detection of transfection efficiency: Human breast cancer cell lines (BT-549, MDA-MB-231, and MCF-7) were cultured in 6-well plates, with the cells evenly distributed. After 24 hours of incubation, the cells were transfected when a cell confluency of 60% was reached. 48 hours after transfection, Total RNA was isolated using the TRIzol reagent and real-time PCR was performed using an RT-qPCR kit to determine the relative expression of LncRNA FOXD3-AS1 so as to ascertain the transfection efficiency.

1.4.3 Cell proliferation ability：2×10^3 cells were plated in 96-well plates and transfected 24 hours later. After transfection, the cells were cultured for 0, 24, 48,72, and 96 hours, respectively. According to the CCK-8 manual, the absorbance (OD) of each well was measured, and the experiment was performed in triplicate.

1.4.4 Wound healing assay : The cells were seeded in 6-well plates and transfected with si-NC or si1 when a confluency of 70% was reached. Next, a 200 μL gun head from NEST Company was used to draw a line, cleaned with PBS, and a specific position was selected under the microscope as the 0h control. The corresponding positions were photographed again after 24 hours and 48 hours, respectively, and the change in the scratch area was measured.

1.4.5 Transwell migration assay: The transfected BT-549 and MDA-MB-231 cells were collected, and a 200 μL cell suspension containing 3×10^4 cells was added to the upper chamber of the Transwell chamber, while a 600 μL medium containing 20% fetal bovine serum was added to the lower chamber. After being cultured in an incubator at 37 ℃ for 24 hours, the unmigrated cells in the upper chamber were gently wiped with cotton swabs, fixed with methanol for 30 minutes, and then stained with Crystal violet dye (Biyuntian Biotechnology) for 15 minutes. The number of cells passing through the Transwell chamber was observed under a 10×10-fold microscope, and 4 visual fields were randomly selected, and images were taken. The experiment was repeated in triplicate, and the average number of cells was calculated and evaluated using graphs.

1.4.6 Transwell invasion assay: The Matrigel glue (Corning, USA) was diluted with a serum-free medium at 1:5. 100 μL of the solution was added to the upper chamber of Corning's Transwell chamber and placed in a 37 ℃ incubator for solidification. Approximately 3×10^4 transfected BT-549 and MDA-MB-231 cells were suspended in a 100 μL serum-free medium and added to the upper chamber of the Transwell chamber. Afterward, a 600 μL medium containing 20% fetal bovine serum was added to the chamber. The method for observation and analysis was identical to that of the Transwell migration assay.

1.4.7 Detection of apoptotic ability: According to the manufacturer's instructions, Annexin Ⅴ-FITC and PI were mixed with the transfected cells and detected by flow cytometry.

1.4.8 Western blot: The transfected cells were washed with PBS 3 times, then the RIPA lysate containing various protease inhibitors was added, and the cells were scraped off. After 20 minutes of lysis on ice, the solution was mixed, centrifuged at 4 ℃ and 12000 r/min for 20 minutes, and the supernatant was subsequently collected. After being quantified by the BCA protein quantitative kit (Biyuntian Biotechnology), the protein was mixed with 4 × loading buffer at a ratio of 1:3. The protein sample was

heated for 10 minutes, and the sample volume was calculated according to the standard curve. The samples were separated by 8% polyacrylamide gel electrophoresis at a voltage of 60 V, followed by electrophoresis at an adjusted voltage of 90 V, and then transferred to PVDF membranes. Then, the membranes were blocked with 5% skimmed milk for 1 hour, washed with PBST 3 times, and incubated with the primary antibodies, namely, GAPHD and β-Catenin at 4 ℃ overnight. After washing three times with PBST, the membranes were incubated with the HRP secondary antibody for 1 hr at room temperature. Finally, the bands were developed using a chemiluminescence kit (Pierce, USA).

1.5 Statistical methods: Statistical analyses were performed by the SPSS 26.0 statistical software. All data were expressed as mean ±standard deviation. Comparison between two groups was determined by the t-test, and a p-value less than 0.05 was considered statistically significant.

2. Results

2.1 FOXD3-AS1 is highly expressed in breast cancer cell lines

Results from the TCGA database analysis showed that the expression of LncRNA FOXD3-AS1 in breast cancer was significantly higher than that in adjacent tissues (Fig. 1a). Therefore, GAPDH was used as the internal control. Moreover, results from the RT-qPCR assay demonstrated that the relative expression of LncRNA FOXD3-AS1 in human breast cancer cell lines BT-549, MDA-MB-231, and MCF-7 (Fig. 1b) was 10.37 ±0.76, 6.94 ±0.88, and 3.77 ±0.42, respectively. Interestingly, the relative expression of FOXD3-AS1 in the normal human breast epithelial cell line was significantly higher than that in the normal human breast epithelial cell lines (all $P < 0.01$). Given that the expression of LncRNA FOXD3-AS1 was highest in the BT-549 and MDA-MB-231 cells, these two cell lines were selected for subsequent experiments.

2.2 The effect of knocking down the expression of LncRNA FOXD3-AS1 on the biological behavior of cells

In human breast cancer cells, BT-549 and MDA-MB-231 with high expression of LncRNA FOXD3-AS1.Three siRNAs targeting LncRNA FOXD3-AS1 were transfected in BT-549 (Fig. 1c) and MDA-MB-231 (Fig. 1d) cells. The knockdown efficiency of LncRNA FOXD3-AS1 was detected by RT-qPCR. The siRNA sequence is summarized in the table below. The results determined that the si1 and si2 successfully knocked down the expression of LncRNA FOXD3-AS1. Therefore, si1 and si2 were selected for the ensuing experiments.

2.2.1 The effect of LncRNAFOXD3-AS1 on the proliferation ability of breast cancer cells: The results from the CCK8 assay (Fig. 2a-b) showed no significant difference in the proliferative ability of breast cancer cells compared with the control group.The results of the wound healing assay (Fig. 2c-d) demonstrated that the cells in the si1 group approached the scratch area more slowly than those in the control group.

2.2.2 Effect of LncRNA FOXD3-AS1 on migration and invasion ability of breast cancer cells:

The results of the wound healing assay (Fig. 2c-d) demonstrated that the cells in the si1 group approached the scratch area more slowly than those in the control group. Results from the Transwell migration assay (Fig. 3a-b) revealed that the number of transmembrane cells in the si1 and si2 groups was lower than that in the siNC group (464.7 ±46.8). Furthermore, in MDA-MB-231 cells, the number of transmembrane cells in the si1 and si2 groups was lower than that in the siNC group ($P < 0.001$). Transwell invasion assay (Fig. 3c-d) results revealed that the number of transmembrane cells in the si1 and si2 groups was lower than that in the siNC group ($P < 0.0l$). In MDA-MB-231 cells, the number of transmembrane cells in the si1 (111.3 ±1.52 cells) and si2 groups (114.3 ±7.5 cells) was lower than that in the siNC group ($P < 0.001$). These results suggest that LncRNA FOXD3-AS1 can inhibit the migration and invasion of breast cancer cells.

2.2.3 Effect of LncRNA FOXD3-AS1 on the apoptosis of breast cancer cells: The results from flow cytometry (Fig. 4a-b) exposed that the apoptotic rate of BT-549 cells in the si1 (23.4% ±6.5%) and si2 groups (29.1% ±8.6%) was higher than that in the siNC group (17.4% ±7.4%). Likewise, the apoptotic rate of MDA-MB-231 cells in the si1 (12.1 ±4.6%) and si2 groups (16.9 ±7.4%) was higher than that of the siNC group (6.4% ±3.8%). The above results indicate that the apoptotic rate of the si1 group was higher than that of the control group, but the difference was not statistically significant.

2.3 Effect of LncRNA FOXD3-AS1 on the Wnt/β-Catenin signal pathway: In BT-549 and MDA-MB-231 cells, the level of the β-Catenin protein in the si1 and si2 group was significantly lower than that in the siNC group (Fig. 4c-d).

3. Discussion

Breast cancer is one of the most common malignant tumors in women, has undoubtedly become the biggest threat to women's health. At present, early diagnosis and treatment are pivotal to the survival of breast cancer patients. Therefore, the search for effective biomarkers is crucial for the prevention and treatment of breast cancer. The factors that impacting the survival of breast cancer patients are primarily the recurrence and metastasis , which is also a challenge to reduce the mortality of the disease. A number of past and ongoing studies have demonstrated the significance of LncRNAs in the occurrence and development of diseases[14]. LncRNAs are important universal genes that play a regulatory role in almost every stage of gene expression. Anomalous expression of certain lncRNAs mediates a range of biological functions, such as, cell proliferation, metastasis, invasion, apoptosis, cell cycle arrest, inflammation, and even osteogenic differentiation. Aberrant expression of lncRNAs may be a predictor of poor prognosis in malignant tumor[15]. With the rapid development of high-throughput sequencing technology, the number of newly identified and unidentified LncRNAs have considerably increased. In the study of Li et al[16], a number of 696 differentially expressed LncRNAs were found, and they were analyzed by monofactor analysis and multifactor Cox regression analysis. Twelve lncRNAs were identified as prognostic markers for breast cancer. The study by Ma et al[17], found that the expression of LNCRNA Linp1 increased significantly in breast cancer patients, and knocking down the expression of LNCRNA LINP1 abated the resistance of tamoxifen-resistant breast cancer cells.

It has been confirmed that LncRNA FOXD3-AS1 is highly expressed in several malignant tumors (such as breast cancer, lung cancer, cervical cancer, nasopharyngeal carcinoma, etc.). Inhibiting its expression considerably reduces the ability of malignant biological behaviors such as proliferation, migration, and invasion of tumor cells. Here in, after the inhibition in the expression of LncRNAFOXD3-AS1, the migration and invasion abilities of breast cancer cells BT-549 and MDA-MB-231 greatly decreased. Nevertheless, proliferative and apoptotic abilities were not significantly altered. Current studies have confirmed that in most malignant tumors, a high expression of LncRNA FOXD3-AS1 indicates a high degree of malignancy and poor prognosis. The use of targeted interference RNA technology can target inhibit the expression of LncRNA FOXD3-AS1, which is expected to inhibit the progression of malignant tumors. Besides, previous studies have established that LncRNA FOXD3-AS1 can play its role as a mechanism for ceRNA[18] to regulate gene expression, PARP1, CTCF, FOXD3 mRNA, protein levels, and numerous signaling pathways, but the specific mechanism is unclear and warrants further investigations. There are many signal pathways related to breast cancer, such as the Wnt/β-Catenin signal pathway, TGF-β/Smads pathway, AKT pathway, and so on. The Wnt/β-Catenin signaling pathway is instrumental in embryonic development and maintaining adult organs and tissues. WNT is a secretory protein that binds to cell surface receptor to activate downstream signaling cascades. The normal Wnt signaling pathway is closely related to human development and homeostasis[19]. β-catenin is a key factor in Wnt/β-catenin signaling pathway and a potential target forthe accurate treatment of malignant tumors. The atypical activation[20, 21] of the Wnt/β-Catenin signaling pathway reduces the degradation of β-Catenin, resulting in an increase in the transcription of downstream target genes (c-myc and CyclinD1), thereby promoting tumor proliferation, migration, and invasion. This study determined that the inhibition of the expression of LncRNA FOXD3-AS1 resulted in the down-regulation of β-Catenin.

To sum up, LncRNA FOXD3-AS1 may enhance the migration and invasion ability of breast cancer cells through the Wnt/β-Catenin signal pathway.

Declarations

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Authors' contributions

BJT did the experiment and wrote the manuscript, YLW, JDL contributed substantial advice help to polish the language, ZJ, ZQX, WTZ, JY, LHZ made diagrams;YZW conducted the project and revised the whole manuscript.All authors read and approved the fnal manuscript.

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Availability of data and materials

The authors confrm that the data supporting the fndings of this study are available within the article.

Ethics approval and consent to participate

This research was approved and supervised by the Hospital Ethics Committee of the Afliated

Hospital of Guangdong Medical University.

Consent for publication

All authors consent to publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Cao W, et al. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. Chin Med J (Engl). 2021;134(7):783–91.
2. Henley SJ, et al., *Annual report to the nation on the status of cancer, part I: National cancer statistics.* Cancer, 2020. **126**(10): p. 2225–2249.
3. Hulvat MC. Cancer Incidence and Trends. Surg Clin North Am. 2020;100(3):469–81.
4. Smittenaar CR, et al. Cancer incidence and mortality projections in the UK until 2035. Br J Cancer. 2016;115(9):1147–55.
5. Bridges MC, Daulagala AC, Kourtidis A. *LNCcation: lncRNA localization and function*. J Cell Biol, 2021. 220(2).
6. Qian X, et al. Revealing lncRNA Structures and Interactions by Sequencing-Based Approaches. Trends Biochem Sci. 2019;44(1):33–52.
7. Tan YT, et al. LncRNA-mediated posttranslational modifications and reprogramming of energy metabolism in cancer. Cancer Commun (Lond). 2021;41(2):109–20.
8. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. Methods Mol Biol. 2016;1402:271–86.
9. Peng WX, Koirala P, Mo YY. LncRNA-mediated Regul cell Signal cancer Oncogene. 2017;36(41):5661–7.
10. Chen Y, Gao H, Li Y. Inhibition of LncRNA FOXD3-AS1 suppresses the aggressive biological behaviors of thyroid cancer via elevating miR-296-5p and inactivating TGF-beta1/Smads signaling pathway. Mol Cell Endocrinol. 2020;500:110634.
11. Zeng ZL, et al. Highly expressed lncRNA FOXD3-AS1 promotes non-small cell lung cancer progression via regulating miR-127-3p/mediator complex subunit 28 axis. Eur Rev Med Pharmacol Sci. 2020;24(5):2525–38.

12. Liu C, et al. LncRNA FOXD3-AS1 Mediates AKT Pathway to Promote Growth and Invasion in Hepatocellular Carcinoma Through Regulating RICTOR. Cancer Biother Radiopharm. 2020;35(4):292–300.
13. E Z, Li C, Xiang Y, *LncRNA FOXD3-AS1/miR-135a-5p function in nasopharyngeal carcinoma cells.* Open Med (Wars), 2020. **15**(1): p. 1193–1201.
14. Chen Y, et al. Long non-coding RNAs: From disease code to drug role. Acta Pharm Sin B. 2021;11(2):340–54.
15. Ma Y, et al. Membrane-lipid associated lncRNA: A new regulator in cancer signaling. Cancer Lett. 2018;419:27–9.
16. Li X, et al. Identification and validation of stemness-related lncRNA prognostic signature for breast cancer. J Transl Med. 2020;18(1):331.
17. Ma T, et al. LncRNA LINP1 confers tamoxifen resistance and negatively regulated by ER signaling in breast cancer. Cell Signal. 2020;68:109536.
18. Xu J, et al. Roles of miRNA and lncRNA in triple-negative breast cancer. J Zhejiang Univ Sci B. 2020;21(9):673–89.
19. Lento W, et al., *Wnt signaling in normal and malignant hematopoiesis*. Cold Spring Harb Perspect Biol, 2013. 5(2).
20. Wang CH, et al. Long non-coding RNA BLACAT1 promotes cell proliferation, migration and invasion in cervical cancer through activation of Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci. 2018;22(10):3002–9.
21. Head JR, et al. Activation of canonical Wnt/beta-catenin signaling stimulates proliferation in neuromasts in the zebrafish posterior lateral line. Dev Dyn. 2013;242(7):832–46.

Figures

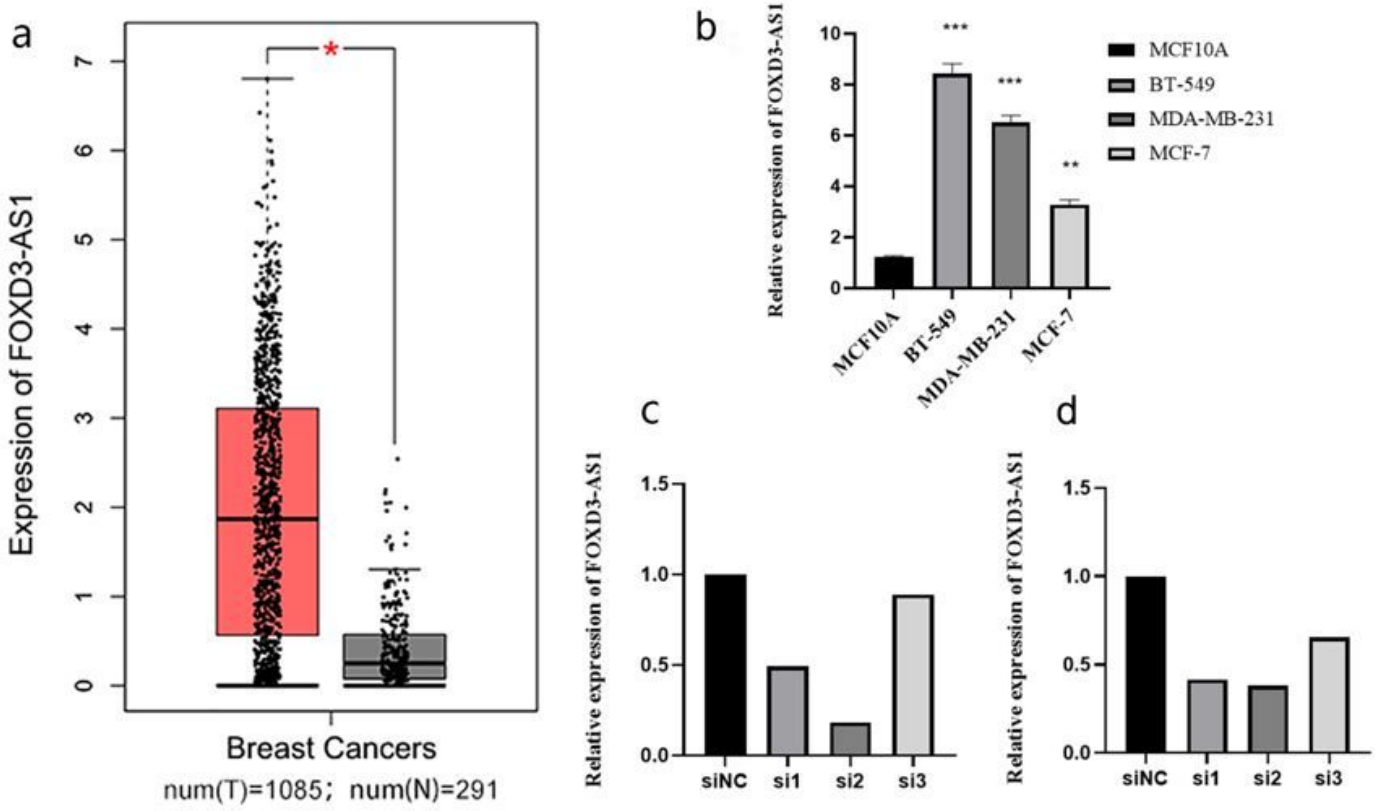

Figure 1

The relative expression of FOXD3-AS1 in breast cancer tissue and breast cancer cells. **a** FOXD3-AS1 was overexpressed in BC samples from The Cancer Genome Atlas. **b** The relative expression of FOXD3-AS1 in the normal human breast epithelial cell line (MCF10A) and three human breast cancer cell lines (BT-549, MDA-MB-231, and MCF-7). **c** The relative expression of FOXD3-AS1 in BT-549 cells after transfection of siNC and three siRNAs targeting LncRNA FOXD3-AS1. **d** The relative expression of FOXD3-AS1 in MDA-MB-231 cells after transfection of siNC and three siRNAs targeting LncRNA FOXD3-AS1.
*P<0.05,**P<0.01,***P<0.001

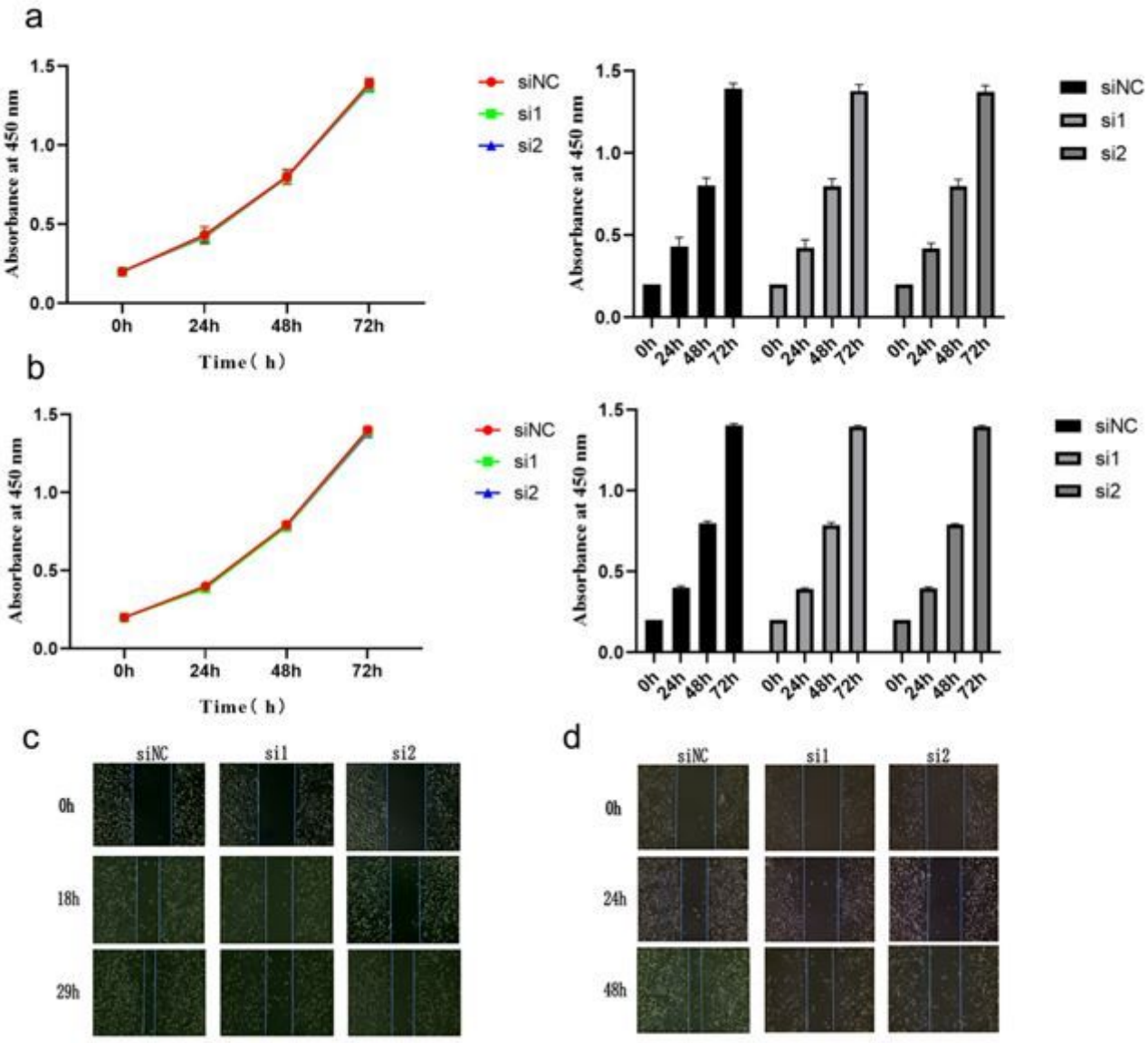

Figure 2

Effect of LncRNA FOXD3-AS1 on proliferation and wound healing ability of breast cancer cells. **a** CCK8 assay were performed to test proliferation ability of BT-549 cells after transfection of siNC, si1 and si2. **b** CCK8 assay were performed to test proliferation ability of MDA-MB-231 cells after transfection of siNC, si1 and si2. **c** Wound healing assay were performed to test wound healing ability of BT-549 cells after transfection of siNC, si1 and si2. **d** Wound healing assay were performed to test wound healing ability of MDA-MB-231 cells after transfection of siNC, si1 and si2.

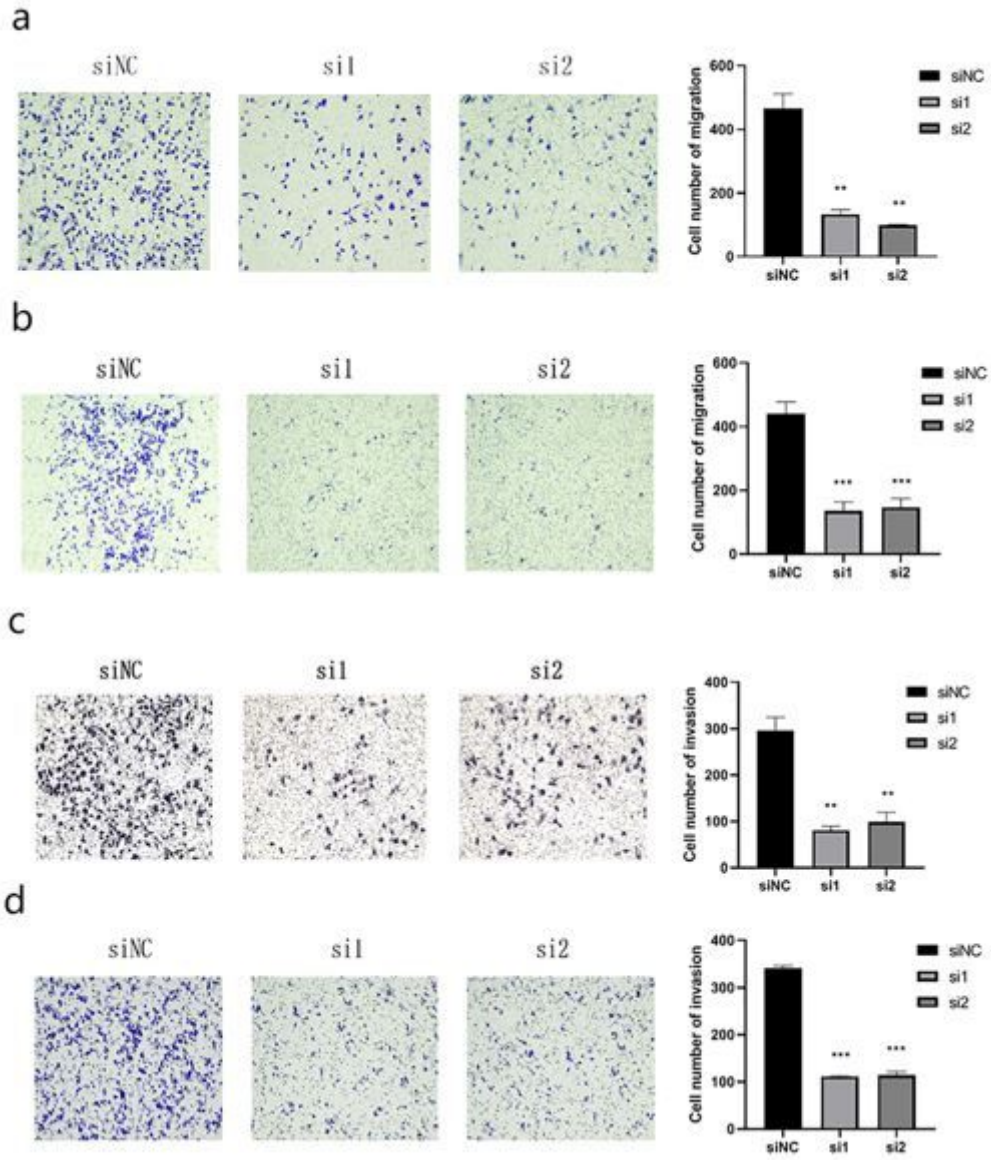

Figure 3

Effect of LncRNA FOXD3-AS1 on migration and invasion of breast cancer cells. **a** LncRNA FOXD3-AS1 enhances the migration ability in BT-549 cells. **b** LncRNA FOXD3-AS1 enhances the migration ability in MDA-MB-231 cells. **c** LncRNA FOXD3-AS1 enhances the invasion ability in BT-549 cells. **d** LncRNA FOXD3-AS1 enhances the invasion ability in MDA-MB-231 cells. **P<0.01, ***P<0.001.

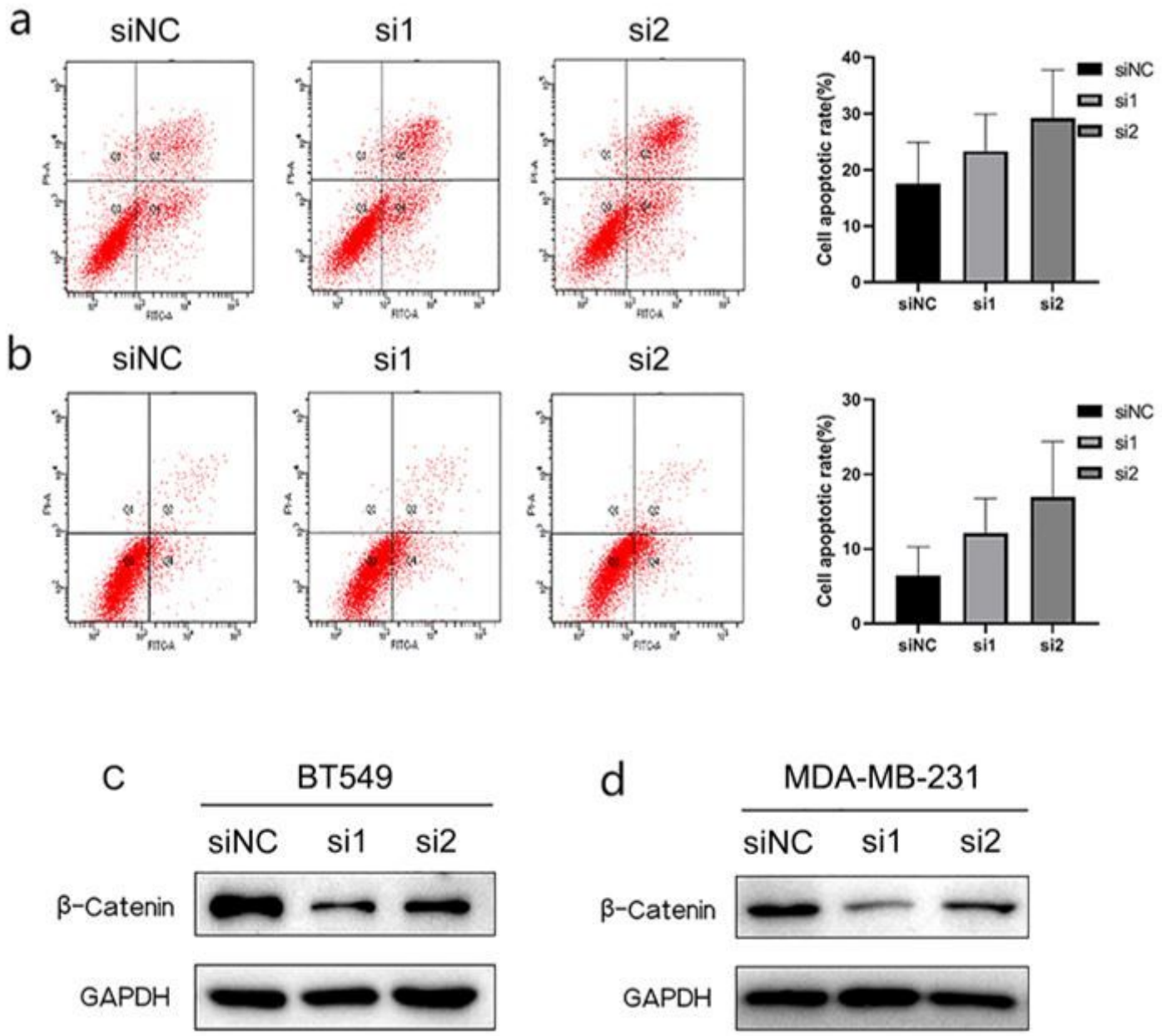

Figure 4

Effect of LncRNA FOXD3-AS1 on the apoptosis and the level of the β-Catenin protein of breast cancer cells. **a** Effect of LncRNA FOXD3-AS1 on the apoptosis in BT-549 cells. **b** Effect of LncRNA FOXD3-AS1 on the apoptosis in MDA-MB-231 cells. **c** Effect of LncRNA FOXD3-AS1 on the level of the β-Catenin protein in BT-549 cells. **d** Effect of LncRNA FOXD3-AS1 on the level of the β-Catenin protein in MDA-MB-231 cells.